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PROMOTER WHICH ALLOWS TRANSGENE EXPRESSION IN THE ENTIRE PLANT EXCEPT IN THE SEED

The present invention relates to the isolation and characterization of a promoter which allows transgene expression in the adult plant, for the purposes of improving the development of the plant, without the product of this transgene being present in the mature and dry seed. The invention also relates to the transgenic plants comprising a gene of interest fused to said promoter sequence.

Molecular biology techniques currently make it possible to modify the genetic inheritance of plants in order to change the components thereof which control production, quality or health. The specificity of expression of the transgenes introduced is essentially based on the use of promoter sequences from plants or from microorganisms. The search for specific promoters is therefore of vital importance for plant biotechnology. Seeds constitute an important component of agriculture, as actual seeds, but also in the food industry or the transformation industry. In this respect, the presence of new proteins and products in the seed may pose problems. It therefore appears to be advantageous to have a promoter which is active in all the vegetative tissues but ineffective in the seeds.

The characteristics of the seed will depend on the interactions between the maturation, under the control of a specific genetic program, and environmental conditions which condition, to a large degree, the subsequent production. However, the mechanisms which regulate these phenomena are, for the most part, still not understood. There exists, therefore, a real advantage in maintaining good seed batch quality. Now, the development of transgenic plants poses new problems, in particular related to the expression of

heterologous genes in the seeds of said plants. Specifically, the presence of proteins or of polypeptides in the seeds may have harmful consequences on their ability to germinate or on their quality. In addition, while the population is becoming increasingly used to the idea that edible plants may be genetically modified, edible seeds containing the product of transgenes may not be easily accepted.

Thus, the objective which is the basis of the present invention is to identify a promoter which would allow strong expression of a transgene in all the tissues of the plants except in the seed.

To this effect, promoter trapping, a powerful tool for dissecting developmental processes (Topping and Lindsey, 1995, for review), has been carried out. This strategy is based on the use of a vector for transforming plants, which has, at one of its ends, a reporter gene (most commonly GUS or GFP) without a promoter. If the insertion occurs in a coding region and if the sequence of the reporter gene is in frame, there will be translational fusion between the endogenous protein and the protein of the marker gene. Gene trapping strategies have a major advantage compared to conventional insertional mutagenesis since the phenotype (expression of the GUS reporter gene) is dominant. This dominance of the phenotype (GUS) makes it possible to follow mutated alleles in the heterozygous state. This is very advantageous for studying mutations which are lethal in the homozygous state. This approach also makes it possible to characterize a gene by its expression.

It has been found, while accomplishing the present invention, that insertion of a reporter gene into the gene encoding a protein of the fatty acid hydroxylase (FAH) type of Arabidopsis leads to expression in all the tissues of the plant except in the seed. This type

of promoter is of great value for biotechnological applications. It makes it possible to express a protein of interest as soon as impregnation occurs in all the tissues of the plant, with a high level of expression, except in the seed. It is therefore possible, for example, to protect the plant against many biotic or abiotic stresses without modifying the content of its seed. It is also possible to express an antisense sequence directed against a target gene in all the tissues except in the seed.

Description

Thus, the present invention relates to a promoter sequence which allows the expression of a gene of interest in the tissues of a plant except in the maturing seed and in the dry seed, said sequence comprising a sequence having at least 80% identity with the sequence, or a portion of the sequence, of the promoter of the Arabidopsis FAH gene.

Preferably, this sequence comprises a sequence having at least 80% identity with the sequence, or a portion of the sequence, SEQ ID No. 1.

The term "% identity" is intended to mean the percentage of identical nucleotides, which can be easily calculated by those skilled in the art using a sequence comparison computer program, such as the DNASIS program (Version 2.5 for Windows; Hitachi Software Engineering Co., Ltd, South San Francisco, CA), using the standard parameters described in the manufacturer's manual, incorporated into the description by way of reference.

In this context, the sequences and the percentage identities may also be obtained using internet computer sources. Mention may be made of the Blast program (WWW.ncbi.nlm.nih.gov) and the FastDB program with the

following parameters: Mismatch penalty 1.00; Gap Penalty 1.00; Gap Size Penalty 0.33; joining penalty 30.0. These algorithms are given in Current Methods in Sequencing and Synthesis Methods and Applications, pages 127-149, 1988, Ala R. Liss, Inc., incorporated into the description by way of reference.

The sequences having 80% identity may also be defined as being sequences which hybridize to the sequence SEQ ID No. 1 with high stringency conditions. These conditions are given in Sambrook et al., Molecular Cloning A Laboratory Manual (Cold Spring Harbor Press, 1989) in paragraphs 11.1 to 11.61, incorporated into the description by way of reference.

Advantageously, the sequence according to the invention has the sequence, or a portion of the sequence, SEQ ID No. 1 below:

5'cagctgtagcatcttgatattgctgatactcagccacaagatcgttcatgttactc
tctgcttcattaaactccatctcgtccattccttcttctgtgtaccaatgcaagaaag
cttatctcaacatcaggctgatataaccaatatcttacttcttttacatttgtgaaat
ggaaccaacccatttttctggaaaaagtgctaaccaaacatttgattaaccgtatcac
tactactttcatttctatcttctgtttcattatgctgactatttaagctccgttgtca
aatctctaagttagacataaaagacaaagactaatcaattgtcatcacaccagcgtcg
tcgagtgagctatattaatcgtggatttaagcattaaagaaacattctatagtacta
aagcaaataaaataattataatcaaacactatgcttgacactggtcacgtgtactggt
agtgaatgattctacatcataagaggccgcatcaaaatcctaaaaataagcataatga
attaatcatttacaaattttattttactcaataagaaaatcgaaagtatgattattat
ctagctgccacaatcttcgaatttaatatttactcaagaagagaccgactttaatcct
tgactttctcattgctctatggaaaatgattaaagcagtcaataaaatcttttgacat
tgttggcagaagaccaataattcgaagtctaaaatgtaatcgtccacacagtgtatga
gtatcctagtatttttttcttttccatataagttgaatttgtaatatatatagtgta
atgttgtttatttgtggcaacgtacaaaattgggaatcctataagtgcgacgacaagt
gacaagacgaggctatgaacagctaatgtatgaagagagccaaaagagcaacaacctg
gcacag-3'.

The invention also relates to the use of a portion of the sequence SEQ ID No. 1, for identifying fragments capable of promoting the expression of a gene of interest in a plant except in the seed. It is thus possible to define the minimum region of the sequence of the promoter of the FAH gene for ensuring effective expression. In this sense, the promoter may be modified by adding sequences such as enhancers, and/or by deleting nonessential and/or undesired regions. The promoter may comprise synthetic and/or natural sequences.

The invention relates to a method for isolating and characterizing the promoter of the FAH gene in plants, comprising the following steps:

a) using a primer comprising a sequence having at least 80% identity with a sequence containing at least 10 consecutive nucleotides of the sequence SEQ ID No. 5 or a complementary sequence, or a primer which hybridizes under high stringency conditions to any coding sequence for SEQ ID No. 4 or a sequence having at least 80% identity with a sequence containing at least 10 consecutive nucleotides of the genomic sequence of the FAH gene of Arabidopsis, accessible under the number AC003096, or a complementary sequence, for isolating and/or amplifying the sequence upstream of the 5' end of the FAH gene,

b) cloning and sequencing of the sequence obtained in step a).

SEQ ID No. 5 corresponds to the coding sequence of the FAH gene of Arabidopsis:

DEFINITION: complete cDNA of Arabidopsis thaliana fatty acid hydroxylase Fah1p (FAH1)
ACCESSION: AF021804
ORGANISM: Arabidopsis thaliana, Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;

Euphyllophytes; Spermatophyta; Magnoliophyta; Eudicotyledons; Rosidae; Brassicales; Brassicaceae.

Reference: Mitchell, A.G. and Martin, C.E, (1997). Fah1p, a saccharomyces cerevisiae cytochrome b5 fusion protein, and its arabidopsis thaliana homolog that lacks the cytochrome b5 domain both function in the alpha-hydroxylation of sphingolipid-associated very long chain fatty acids; J. Biol. Chem. 272 (45), 28281-28288 MEDLINE 98019193

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  1 atggttgctc agggattcac tgtggatctt aaaaagcccc ttgtatttca ggttggtcat
 61 cttggagaag attatgagga atgggttcac caacctatcg cgaccaagga aggccctcgg
121 tttttcaga gtgactttg ggagttcttg acacttacag tttggtgggc agttcctgtc
181 atttggttgc cagttgtagt ctggtgcata tcaaggtcag taagtatggg atgttcactt
241 ccagaaatcg tcccaattgt tgtcatggga atattcatct ggacattttt tgaatacgtt
301 cttcaccggt tcgttttcca cataaaaacg aagagttact ggggaaacac tgcacactat
361 cttattcacg gatgccatca taagcacccg atggaccacc ttcggctcgt ctttcctcct
421 actgcaactg cgattttatg ctttccgttc tggaacattg cgaaggctat ctcaactcct

481 tcaaccgcac ctgcattgtt tggtggaggc atgctcggat atgtgatgta cgatgtcact
541 cattattacc ttcaccatgc ccaacctact agaccagtga ccaaaaatct caagaagtac
601 catttgaatc atcacttcag gattcaggac aaaggatttg gtataacttc gtcgttatgg
661 gacatagtct ttgggacact tcccaccaca aaagccccca gaaaagagca atag
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It is also possible to use a primer comprising a sequence having at least 80% identity with a sequence having at least 10 consecutive nucleotides of the genomic sequence of the Arabidopsis FAH gene (introns and exons) which is accessible to those skilled in the art under the number AC003096, or a primer which hybridizes, under high stringency conditions, to any coding sequence for the following SEQ ID No. 4 (Arabidopsis thaliana, fatty acid hydroxylase Fah1p):

MVAQGFTVDLKKPLVFQVGHLGEDYEEWVHQPIATKEGPRFFQSDFWEFLTL
TVWWAVPVIWLPVVVWCISRSVSMGCSLPEIVPIVVMGIFIWTFFEYVLHRFVF
HIKTKSYWGNTAHYLIHGCHHKHPMDHLRLVFPPTATAILCFPFWNIAKAISTP
STAPALFGGGMLGYVMYDVTHYYLHHAQPTRPVTKNLKKYHLNHHFRIQDK
GFGITSSLWDIVFGTLPTTKAPRKEQ

Thus, the promoter sequence which allows expression of a gene of interest in the tissues of a plant, except in the maturing seed and in the dry seed, may also be characterized in that it comprises a sequence which has at least 80% identity with the sequence, or a portion of the sequence, of the promoter of the FAH gene, and which can be obtained using the method described above.

Another aspect of the invention relates to an expression cassette which comprises a sequence of interest fused to a sequence comprising a promoter sequence as defined above. Said sequence of interest may encode an RNA, a protein or a polypeptide which protects the plant against a biotic or abiotic stress.

The cassette may allow the cosuppression of the expression of a gene, characterized in that said sequence of interest encodes a protein or polypeptide capable of substituting the function of an endogenous protein or polypeptide. The sequence of interest may also encode an antisense sequence directed against a target gene. This makes it possible, in coupling with the ectopic overexpression of a gene of interest in the seeds, or preventing expression of this gene in other tissues, the antisense not being expressed in the seeds. This proves to be most useful when the desire is to overexpress a protein in the seeds without disturbing the development of other tissues of the plant.

The cassette according to the invention may also comprise a selection marker gene, a leader sequence which controls the transit, the secretion or the targeting of the expression product, in various organelles, a transcription termination signal sequence and a translation termination signal sequence.

In the context of the invention, the term "gene of interest" or "transgene" is intended to mean a gene in particular selected from the genes encoding a protein or a polypeptide which protects the plant against a biotic or abiotic stress, the disturbing genes encoding a product capable of substituting for and/or inhibiting the function or the expression of an endogenous mRNA, protein or polypeptide. Mention may be made, for example, of the genes encoding ribozymes against endogenous mRNAs, and genes, the transcription product of which is at least in part complementary to an endogenous target mRNA (EP 240 208, incorporated into the description by way of reference). Mention may also be made of genes, the transcription product of which is identical or similar to the transcripts of endogenous genes, which are capable of inhibiting by cosuppression the expression of said endogenous genes (Napoli C. et al., 1990, The Plant Cell, 2, 279-289 mentioned in the description by way of reference). Of course, the gene according to the invention may encode an enzyme involved in metabolism, so as to produce or promote the biosynthesis of metabolites, in particular of metabolites which are useful for the human or animal diet or which may affect development. The promoter sequence according to the invention may induce the expression of a foreign gene and be used in various types of plant. The term "foreign gene" or "transgene" is also understood to define any coding or noncoding region of DNA (protein, polypeptide, antisense, catalytic RNA, viroid, etc.). A protein of interest for the development and production of the plant may be produced constitutively in all the organs of the plant

using this promoter, without the composition of the seed being effected. The proteins of interest are, without this being an exhaustive list, those which allow better protection of the plant against

- biotic stresses: protection against pathogens, bacteria, fungi, insects, nematodes, parasites or ravages, protection against intracellular pathogens and viruses, in particular those which are not transmitted by the seeds;
- abiotic stresses: protection against heat and cold, frost, water-related stresses such as drought or the opposite, anoxia, pollution (ozone, SO_2), photoinhibition and light stresses, beating down, phytoremediation or nutritional stresses caused by a deficiency or excess of a nutrient element (in particular a saline stress).

Any gene of interest may therefore be placed under the control of the isolated promoter sequence. For expression in plants, this gene may also comprise 3' nontranscribed sequences containing polyadenylation signals which are active in plants. These sequences may, for example, be those encoding the 3' transcribed, untranslated portion of the cauliflower mosaic virus 35S RNA gene (CaMV 35S) or the 3' untranslated region of the gene encoding the nopaline synthase (NOS) of the Agrobacterium tumefaciens Ti plasmid.

The gene of interest according to the invention may also be a gene which controls development, such as for example a gene involved in hormone metabolism, in signal transduction or in the control of the cell cycle.

Another aspect of the invention relates to a vector, in particular a plasmid vector, comprising an expression cassette as defined above.

A subject of the invention is also a plant cell transformed with the cassette or with a vector comprising said cassette, and a plant transformation kit comprising said cassette or said vector.

The plasmid preparation, the chimeric gene and expression cassette construction, the DNA restriction using endonuclease, the transformation and the confirmation of transformations are carried out according to standard protocols (Sambrook et al. 1989, Molecular Cloning Manual Cold Spring Harbor Laboratory, incorporated into the description by way of reference).

The construction of the vectors which can be used for the transformation experiments forms part of the known molecular biology techniques carried out routinely in this field of use.

An additional aspect of the invention relates to a method for preparing transgenic plants in which a gene of interest is expressed in all the tissues except in the maturing seed and in the dry seed, characterized in that it comprises the following steps:

a) transferring a cassette or a vector according to the invention into plant cells,

b) culturing the transformed cells obtained in step a) so as to obtain said transgenic plants.

The DNA may be transferred into the plant cells, in particular the cells of the albumen or the totipotent cells derived from immature embryos, using standard techniques (Plant Cell Report, 10, 595, 1992), in particular by transfer via Agrobacterium (Plant J., 1994, 6, 271), by electroporation (Nature, 1987, 327, 70) or laserporation (Barley Genetics, 1991, VI, 231), with polyethylene glycol, or using the "particle gun" biolistic method (Nature 1987, 327, 70). In general, for the vectors for transformation via an agrobacterium (infiltration in planta Bechtold et al. 1993), the

transformation vectors carry selection markers, T-DNA borders, cloning sites, replication functions and other elements so necessary for good transgene transfer (Bouchez et al. 1993). The publications mentioned above are incorporated into the description by way of references.

A subject of the present invention is also a transgenic plant which can be obtained by carrying out the method mentioned above.
The expression "plant which can be obtained" is intended to mean any plant expressing a transgene in its tissues except in the mature and dry seeds, said plant containing a promoter according to the invention. The plants obtained by any equivalent method leading to the same results are also a subject of the invention. The list of plants in which this promoter sequence may be used includes more particularly the plants which are useful for any industry. Mention may be made, for example, of rapeseed, crucifers, maize, soybean, wheat, sunflower, pea, ornamental plants and trees.

Thus, the invention relates to a plant, as defined above, which expresses in its tissues, except in the seeds, a gene, the product of which (RNA or protein) protects the plant against a biotic or abiotic stress, an antisense sequence directed against a target gene, a protein or polypeptide capable of substituting for the function of an endogenous protein or polypeptide, or a coding sequence for a protein involved in metabolite biosynthesis or a gene which controls development, such as for example a gene involved in hormone metabolism, in signal transduction or in the control of the cell cycle. The plant according to the invention may also express a protein of interest under the control of a promoter other than the promoter of the FAH gene and an antisense sequence capable of inhibiting the expression of said protein of interest under the control of the

promoter of the FAH gene, such that the gene of interest is expressed only in the seeds.

The seeds obtained from a transgenic plant according to the invention, which therefore do not contain the product of expression of the transgene, are targeted by the present invention, as is their use in any industry.

For the remainder of the description, reference will be made to the legends of the figures presented below.

Legends

Figure 1: Intron/exon structure of the mRNA of the FAH gene

The rectangles with stripes represent the introns.
The scale is given on the figure.
T29F13 is a bac and TAI234 is a cDNA.

Figure 2: Structure of the [lacuna] region of the FAH gene

PFAH upper and A1 represent the primers used to sequence the promoter.
The rectangles with the stripes represent the 5' transcribed, untranslated portion.
The scale is given on the figure.

Figure 3: Map of the pBI 101 plasmid

Map of the pBI101 plasmid containing the pFAH promoter used.

Example 1: Cloning of the promoter

Materials and methods

Isolation of the promoter region of FAH

The method used for the extraction of Arabidopsis genomic DNA is based on that described by Doyle and Doyle (1990). The principle is based on the detergent

properties of cetyltrimethylammonium bromide (CTAB; Sigma Chemical Co., USA) which allow the specific denaturation of protein and polysaccharide macromolecules. Approximately 2 g of plant material (plantlets cultivated in vitro, 1 to 2 weeks old) are finely ground in liquid nitrogen and transferred into a 50 ml tube of the FALCON type (Costar, USA), containing 7.5 ml of extraction buffer preheated to 65°C. The extraction is carried out at 65°C for 30 minutes, with regular stirring. The proteins denatured by the β-mercaptoethanol and the CTAB in the buffer are then extracted in one volume of chloroform, followed by elimination after centrifugation (4430 g, 10 min). The nucleic acids in the supernatant are precipitated with one volume of isopropanol in the presence of 3M sodium acetate (1/10, v/v), centrifuged (7900 g, 10 min) and then rinsed with 70% ethanol. The pellet is taken up in an Eppendorf tube in 100 µl of water and the ribonucleic acids are eliminated by adding 3 µl of Rnase A at 10 mg/ml (Sigma Chemical Co., USA). The DNA is deproteinized and then again precipitated with absolute ethanol. After centrifugation in an Eppendorf tube, the pellet is washed, dried, taken up in 50 to 100 µl of water and stored at -20°C before analyses.

Amplification of the genomic DNA

The promoter sequence is amplified using PCR technology, which is a known technique (Sambrook et al. 1989). The primers corresponding to the 5'(upper) and 3'(lower) parts of the promoter sequence were derived from the genomic sequence of BAC T29F13 (AC003096) (see figure 1). Genomic DNA from a wild-type line (Ler) was used as the matrix for amplifying the promoter component. The amplification reactions were carried out on a thermocycler (MJ Research PTC100-96), in 0.2 ml tubes (Prolabo) containing the following mixture:

1 µl (10 ng) DNA, 2 µl 10 x buffer (BRL), 2 µl 25 mM $MgCl_2$, 0.8 µl 5 mM dNTP, 1 µl primer 1 (10 pmol/µl),

1 µl primer 2 (10 pmol/µl), 0.5 µl (1U) Taq DNA polymerase (5U/µl) and H_2O qs for 20 µl.
upper (5'-3'): TTCATGTTACTCTCTGCTTC (SEQ ID No. 2)
lower (5'-3') GGAAAGGAAACAAATACGGATTC (SEQ ID No. 3)

Bacterial transformation

The genotypes of bacteria used for carrying out the experiments are:

E. Coli strain DH12S (φ80, dlaZ ΔM15 mcrA Δ(mrr-hsdRMS-mcrBC) araD139 Δ(ara,leu)7697 ΔlacX74 galU galK rpsL deoR nupG recAl/F'proAB+lacIq ZΔM15).
Agrobacterium tumefaciens pmp90C58CE

The bacteria (E.coli strain DH12S) are transformed with a recombined plasmid by electroporation (Potter, 1993). 2 µl of the ligation reaction are mixed, in an electroporation cuvette (1 ml, width 0.1 cm), with 50 µl of thawed bacteria and kept in ice. The cuvette is then placed in an electroporator (Gene Pulser II System: BIO-RAD, FRANCE) and a voltage of 1.25 kV is applied for a period of time which depends on the resistance (200Ω) and on the capacity (25 µF) of the circuit. One ml of SOC medium is added to promote the growth of the bacteria and the entire mixture is incubated in a 10 ml tube for 2 hours at 37°C, with rotary shaking (220 rpm). The transformed bacteria are then plated out onto dishes containing solid LB medium supplemented with the appropriate antibiotic, and incubated at 37°C overnight. The bacteria transformed with the recombined pMeca plasmid are selected with 0.04 mg/ml of ampicillin in the presence of 0.2 mg/ml of X-Gal and of 0.05 mg/ml of IPTG. For the other recombined plasmids, the bacteria are selected on an LB medium with the appropriate antibiotic at a final concentration of 0.04 mg/ml.

β-Glucuronidase activity

For the seeds, they are sowed onto a double thickness of Whatman 1M paper of 4.7 cm (Maidstone, England) soaked with 2 ml of sterile water. After soaking for 48h in a dish saturated with water, the seeds are scraped off and placed in an Eppendorf tube to which 100 µl of infiltration buffer (100 mM of phosphate buffer, pH 7.2, 10 mM EDTA, 0.1% v/v Triton X100), supplemented with X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) are added. The X-Gluc is dissolved in DMF (dimethylformamide) at a stock concentration: 100µ (10 mg/100 µl). The infiltration buffer is supplemented at 1/100th extemporaneously with the X-Gluc stock. For the other tissues, the samples are placed directly in the infiltration buffer and the coloration is then produced according to the same protocol.

The infiltration is carried out under vacuum (in a vacuum bell jar):

- the vacuum is broken twice.
- the vacuum is maintained for 1 hour, and the samples are then placed at 37°C overnight.

Results

Preliminary analyses indicated that an enzyme involved in lipid metabolism (fatty acid hydroxylase: FAH) may have an expression corresponding to the type of promoter having the desired characteristics.

The sequence of the gene in question was obtained by virtue of the sequences originating from the systematic sequencing of the Arabidopsis thaliana genome, and is located on BACT29F13. An expressed sequence (EST TAI234) was identified in the databases and appears to correspond to a full length sequence of the FAH mRNA. This allowed identification of the 5' transcribed untranslated sequence and of the anticipated positioning of the promoter sequence. The intron/exon structure was deduced, at the level of the

transcribed, untranslated portion, from the alignment of the BAC with EST TAI234 (figure 1).

The promoter was amplified by PCR using the primer pFAH/upper and the primer A1, placed in the 5' transcribed/untranslated portion (figure 2). A study of the sequence showed that the amplified sequence contains a putative TATA box at -100 bp from the presumed transcription initiation site (according to the full length cDNA) and a CCAAT box at -190 bp from this same transcription. The amplified PCR fragment (932 bp) was cloned into a pGEM-T vector (PROMEGA) sequenced, and then introduced into a binary vector (pBI101, Clontech) containing a GUS reporter gene without a promoter (figure 3). This construct was then introduced by transformation in planta, via Agrobacterium, into wild-type plants (ecotype Ws). Thirteen primary transformants were obtained, which were tested for their GUS activity during their development.

Example 2: Expression of the reporter gene under control of the promoter of the FAH gene

In the embryo, the expression is strong from 20 hours after the start of soaking. During development, the expression is strong in all the tissues, with a certain preference for the vascular tissues.

These results demonstrate that the isolated promoter sequence indeed confers a very specific expression profile on the reporter gene used (GUS). The promoter is active throughout the development of the plant, in all the tissues tested (leaves, flowers, stems, roots, etc.) except in the seed undergoing maturation (see Table I below).

Table I: Expression profile for the GUS reporter gene

	Coty-ledons	Adult leaf	Roots	Flower	Siliqua	Germin-ating seeds	Dry seeds
1	++	+++	++	++	+++	+++	-
2	+++	+++	++	++	+++	+++	-
3	+++	+++	++	++	nd	++	-
4	+++	+++	++	++	nd	++	-
5	+++	+++	++	++	nd	+++	-
6	+	+++	++	++	nd	+++	-
7	+++	+++	++	++	+++	+	-
8	++	+++	++	++	+++	+++	-
9	+++	+++	++	++	+++	++	-

The expression of the marker confirms the functionality of the promoter and its specificity. This type of promoter is therefore of very great value for biotechnological applications, such as the expression of an anti-insect toxin (Bt type) in plants and the expression of any transgene making it possible to improve, quantitatively or qualitatively, the development and growth of the plant, without the protein encoded by the transgene being present in the seed.

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